wholly in the higher elevations, primarily on a series of ridges throughout central and south central Connecticut. This is coincident with reports that *M. septendecim* is primarily found in upland woods (Beamer, 1931, Dybas & Lloyd, 1962). In Connecticut, the lowlands and slopes once cleared for agricultural purposes have become reforested, yet there appears to be no noticeable migration of cicadas into these areas. The periodical cicada has a wide range of host plants, and the deciduous flora on the ridges and the valleys does not differ to any great extent. Restriction to the upland habitat by *M. septendecim* (at least in Connecticut) is not due to displacement by *M. cassanii*.

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FREE FAT AND GLYCOGEN DURING METAMORPHOSIS OF *MUSCA DOMESTICA* L.

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Abstract During the metamorphosis of the house fly, both glycogen and fat are used as direct energy sources. At 25°C., the percentage of glycogen decreases from 0.99 in the prepupa to 0.34 in the 2-day pupa. It increases to 0.54 during the next 24 hrs. at the expense of fat and then diminishes to 0.28 in the newly emerged adult. The percentage of fat increases from 8.72 in the 6-day larva to 12.39 in the 1-day pupa. A loss of 2.8% occurs at the time glycogen increases and another loss of 2.6% occurs during the change from the 4-day pupa to the adult. These decreases are greater than required for the production of glycogen and for the loss of fat in the puparium and pupal skin on emergence.

Fats and glycogen are important sources of energy during the metamor-

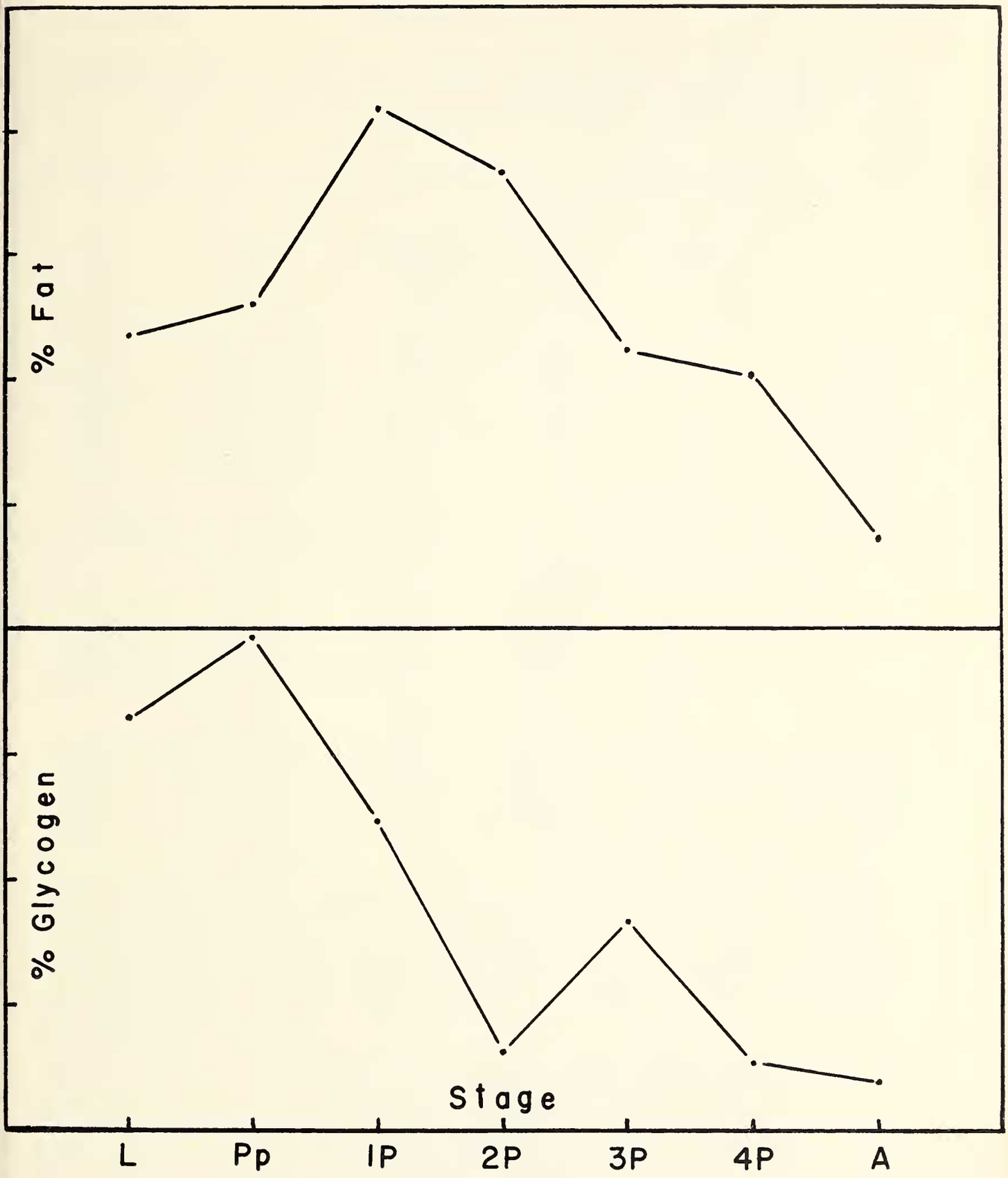
phosis of holometabolous insects. A decrease in fat content during the pupal stage was found by Rudolfs (1926) in the tent caterpillar, *Malacosoma americana;* by Becker (1934) and by Moran (1959) in the mealworm, *Tenebrio molitor;* and by Haub and Hitchcock (1941) in the blow fly, *Phormia regina.* Pearincott (1960) observed that the fatty acid content of the house fly, *Musca domestica,* decreased progressively during this stage. Ludwig and Rothstein (1949) showed that in the Japanese beetle, *Popillia japonica,* glycogen is utilized during the early part of the pupal stage. It is then replenished at the expense of free fat during the fifth day of pupal life at 25°C. The decrease in fat which occurred at this time was sufficient to account for the resynthesis of glycogen. On the other hand, Rousell (1955) observed a steady decrease in glycogen during the pupal stage of the mealworm, *T. molitor.* Moran (1959) stated that in this insect both fat and glycogen furnish the energy for metamorphosis.

This brief review indicates that during the pupal stage the utilization of glycogen may occur in two ways. First, glycogen may serve as a direct source of energy and is synthesized from fats after it reaches a low level (Ludwig and Rothstein, 1949) ; second, there may be a steady utilization of glycogen throughout the pupal stage with no evidence of its resynthesis (Rousell, 1955). The present study was undertaken to determine which type of glycogen utilization occurs in the house fly, and also whether free fats may be used in its synthesis and as a direct source of energy during the metamorphosis of this insect.

Material and Methods

The house flies used in these experiments were of the Wilson strain obtained from Rutgers University. All cultures were kept at 25°C. The adults were fed sugar water and diluted milk, prepared from Borden's Starlac. Eggs were laid on a cotton pad placed in the milk. They were removed within 24 hours after laying and transferred to the larval medium which consisted of Purina dog meal soaked in tap water. Larvae were collected 6 days after hatching. Others were collected just after puparium formation and placed in dated beakers. Insects were used at this stage and are referred to as prepupae. It lasts approximately 24 hours at 25°C. Pupae of known ages, timed within 24 hours, and recently emerged adult flies were also used. All insects were washed with distilled water and then with 65 per cent ethanol for five minutes to remove surface bacteria (Cotty, 1956.) They were then allowed to dry, weighed, and placed under vacuum desiccation where they were allowed to remain until used.

Glycogen determinations were made on groups of five insects by a modified



Explanation of the figure

Figure 1. Changes in the percentages of fat and glycogen during the metamorphosis of the house fly. L, 6-day larva; Pp, prepupa; 1P to 4P, days of pupal life; A, newly emerged adult.

Pflüger technique (Good, Kramer and Somogyi, 1933). The glycogen was then hydrolyzed to glucose by boiling it in 0.6 N HCl for 3 hours in a water bath. The amount of glucose thus formed was measured by the Hagedorn and Jensen procedure (Hawk, Oser and Summerson, 1954, p. 577).

Table 1. Changes in glycogen content during the metamorphosis of the house fly.

Stage	No. of insects	Average wet weight (mg.)	Average weight of glycogen (mg.)	% glycogen with standard errors	"t" value
6-day larva	70	25.23	0.216	0.86 ± 0.019	
Prepupa	70	18.80	0.186	0.99 ± 0.013	5.4
1-day pupa	70	14.68	0.102	0.69 ± 0.024	10.7
2-day pupa	70	14.51	0.050	0.34 ± 0.016	12.1
3-day pupa	70	14.48	0.078	0.54 ± 0.022	6.4
4-day pupa	70	14.15	0.044	0.31 ± 0.016	7.6
Adult	50	11.85	0.033	0.28 ± 0.018	1.3

Table 2. Changes in the free fat content during the metamorphosis of the house fly.

Stage	No. of insects	Average wet weight (mg.)	Average weight of fat (mg.)	% fat with standard errors	"t" value
6-day larva	90	22.59	1.97	8.72 ± 0.28	
Prepupa	84	20.58	1.91	9.28 ± 0.65	0.79
1-day pupa	90	15.09	1.87	12.39 ± 0.99	2.61
2-day pupa	90	16.45	1.86	11.33 ± 1.19	0.69
3-day pupa	90	15.05	1.28	8.50 ± 0.40	2.26
4-day pupa	90	14.95	1.21	8.13 ± 0.42	0.63
Adult	78	12.43	0.68	5.51 ± 0.67	3.40

Fat extractions were made in a Soxhlet apparatus. Six insects were used for each extraction. They were ground in a clean mortar in fat-free sand, purified according to the method of Bloor (1929), and carefully transferred to a fat-free Soxhlet thimble. Free fats were extracted with anhydrous ethyl ether for more than 7 hours. The solvent was then carefully poured into a beaker which had been vacuum desiccated to constant weight. The Soxhlet flask was washed with anhydrous ethyl ether which was then added to that already present in the beaker. The beaker was covered with filter paper and allowed to stand overnight at room temperature. It was then desiccated in a vacuum to constant weight. The difference between the original and final weight of the beaker was used as the milligrams of free fat extracted.

Observations

The percentages of glycogen found in the different stages during the metamorphosis of the house fly are shown in Table 1. There is an increase between the 6-day larva and the prepupa. This increase was followed by a rapid decrease during the next 2 days. An increase, which represents a synthesis of glycogen, occurred between the 2-day and 3-day pupa, followed by a decrease during the last day of pupal life. The table shows that these changes are statistically significant since the difference between the means divided by its standard error ("t" value) is greater than 2 in each case.

The results of the free fat extractions are given in Table 2. There is a synthesis of fat during the transformation from the larval to the pupal stages, followed by a decrease which continues into the adult stage. The increase during early metamorphosis and the decreases which occur between the 2-day and 3-day pupa and between the 4-day pupa and newly emerged adult are statistically significant.

A comparison of the changes in the percentages of fat and glycogen which occur during metamorphosis from the larva to the adult is presented in Figure 1. The graphs show the increase in glycogen and the simultaneous decrease in fat which occurs between the 2-day and 3-day pupa. However, the loss of fat amounts to nearly 3 per cent and the amount of glycogen synthesized to only 0.2 per cent of wet weight.

Discussion

The increase in the percentages of both fat and glycogen observed in the house fly during the changes from the larva to the prepupa and pupa are probably associated with the loss of weight which occurs at this time (Tables 1 and 2). Pearincott (1960) demonstrated that in the house fly there is a simultaneous loss of water so that other constituents become more concentrated. These changes are comparable to those described by Ludwig and Rothstein (1949) for the Japanese beetle, *P. japonica*. However, the decrease in glycogen found during the change from the prepupa to the 1-day pupa, even though a loss of weight and water occurred at this time, suggests its utilization for energy.

The sharp increase in glycogen accompanied by a significant decrease in fat, observed between the 2-day and 3-day pupa, suggests that in the house fly, fats are used in the synthesis of glycogen. In this respect, metamorphosis of the house fly follows the same pattern described by Ludwig and Rothstein (1949) for the Japanese beetle. However, since the loss of fat is much greater than is required for glycogen synthesis, it appears that some of the fat is used as a direct source of energy. The loss of fat which occurs

during the last day of pupal life (4-day pupa to newly emerged adult), which averages more than 2.6 mg., is much greater than can be explained by the loss of the puparium and pupal skin on emergence. Hence, during the metamorphosis of the house fly, both glycogen and fat appear to be used as direct energy sources. In this respect, the results are comparable to those described by Rousell (1955) and by Moran (1959) for the metamorphosis of the mealworm, *T. molitor*.

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